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WO 01/73052 A2

(54) Title: NOVEL THERMOPHILIC POLYMERASE III HOLOENZYME

(57) Abstract: The present invention relates to gene and amino acid sequences encoding DNA polymerase III holoenzyme subunits and structural genes from thermophilic organisms. In particular, the present invention provides DNA polymerase III holoenzyme subunits and accessory proteins of *T. thermophilus*. The present invention also provides antibodies, primers, probes, and other reagents useful to identify DNA polymerase III molecules.

Exhibit B

WO 01/73052

PCT/US01/09950

- 8 -

FIGs. 9A and B. SDS-PAGE analysis of the fraction from the  $\text{Ni}^{++}$ -NTA column purification of N-terminal tagged *T. thermophilus* DnaX.

FIGs. 10A and B. SDS-PAGE analysis of the fraction from the avidin column purification of N-terminal tagged *T. thermophilus* DnaX.

5 FIG. 11. Western analysis of various antiserum dilutions for determination of dilutions to use in *T. thermophilus* DnaX detection.

FIG. 12. Western analysis of various *T. thermophilus* DnaX dilutions for determination of the limit of DnaX detection at antiserum dilution of 1:6400.

10 FIG. 13 The DNA sequence (SEQ ID NO:9) of the *T. thermophilus* *holA* gene ( $\delta$  subunit).

FIG. 14. The amino acid sequence (SEQ ID NO: 10) of *T. thermophilus*  $\delta$ -subunit (*holA* gene).

15 FIG. 15. Alignment of the amino acid sequence of  $\delta$  from *T. thermophilus* and *E. coli*.

FIG. 16 Alignment of the amino acid sequence of  $\delta$ -subunit from *A. aerolicus*, *T. thermophilus*, *B. subtilis*, *E. coli* and *H. influenzae*.

FIG. 17 Biotin blot analysis of growth/induction time optimization of expression of *T. thermophilus*  $\delta$  by pA1-NB-TD/AP1.L1.

20 FIG. 18. Optimization of precipitation of *T. thermophilus*  $\delta$  by ammonium sulfate.

FIGs. 19A and B. SDS-PAGE analysis of fractions from the  $\text{Ni}^{++}$ -NTA column purification of *T. thermophilus*  $\delta$ .

25 FIG. 20. Protein concentration profile of fractions from the avidin column purification of *T. thermophilus*  $\delta$ .

FIG. 21 SDS-PAGE analysis of fractions from the avidin column purification of *T. thermophilus*  $\delta$ .

FIG. 22. The DNA sequence (SEQ ID NO: 16 ) of the *T. thermophilus* *holB* gene encoding the  $\delta'$ -subunit of the *T. thermophilus* Pol III holoenzyme.

WO 01/73052

PCT/US01/09950

13/63

## SEQ ID NO:9

atggtcatogccttcacgggggatcccttctctggcgcgaggccctottagaggaggca  
aggcttaggggcctttcccgccttcaccgagcccaccccgaggccctggcccaggccctc  
gccccggggcttttcgggggccccggggcgatgctggacctgagggagggtgggggaggcg  
gagtgggaaggccctaaagccccctcctggaaagcgtgcccgagggcgccccgtcctcctc  
ctggaccctaagccaagccccctcccgggcgcccttctaccggaaccgggaaaggcgggac  
ttccccaccccccaagggaaggacctggtgcggcacctggaaaaccggggccaagcgctg  
gggctcaggctccccggggcggggtggcccagtaacctggcctccctggagggggacctcgag  
gccttggaacgggagctggagaagcttgcctcctctccctccctcaccctggagaag  
gtggagaagggtggtggccctgaggccccccctcacgggctttgacctggtgcgctccgtc  
ctggagaaggacccccaggaggccctcctgcgcctcaggcgctcaaggaggagggggag  
gagccctcaggctcctcggggccccctctcctggcagttcgccctcctcgccccgggcttc  
ttcctcctccgggaaaacccccaggcccaaggaggaggacctcgcccgctcgaggccac  
ccctacgcgcgcaaaaaggccctggaggcgcgaggcgcttacggaagaagccctcaag  
gaggccctggacgccccctcatggaggcggaagaggcccaagggggggaaagaccatgg  
cttgcctggaggcgggcggtcctccgcctcgccgttga

FIG. 13

The DNA coding sequence of the *T. thermophilus* *holA* gene (SEQ ID NO:9).

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WO 01/73052

PCT/US01/09950

14/63

SEQ ID NO:10

MVIAFTGDFFLAREALLEEARLRGLSRFTEPTPEALAQALAPGLFGGGGAMLDLREVGEA  
EWKALKPILLESVPEGVPVLLLDPKPSPSRAAFYRNRBRDFPTPKGKDLVRHLENRAKRL  
GLRLPGGVAQYLASLEGDLAERERLEKLALLSPPLTLEKVEKVVALRPPLTGFDLVRV  
LEKDPKEALLRLRLKEEGEEPLRLLGALSWQFALLARAFFLLRENPRPKEDLARLEAH  
PYAAKKALEAARRLTEEALKEALDALMEAKRAKGGKDPWLALAAVLRRLAR

FIG. 14

The amino acid sequence of *T. thermophilus*  $\delta$ -subunit.

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